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# Antibacterial and Antifungal Activity of *Thymus serpyllum*

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**Abstract:** In the present study, the hexane, ethyl-acetate, ethanol, butanol, methanol and the aqueous extracts of *Thymus serpyllum* were studied for their antibacterial and antifungal activity. The antimicrobial activity was checked by broth micro dilution method against four bacterial species viz *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus coli*, *Pseudomonas aeruginosa* and four fungal species *Candida albicans*, *Candida parapsilosis*, *Aspergillus fumigatus* and *Aspergillus niger*. The ethyl-acetate and the methanolic extracts were found to be active against all tested bacteria and fungi with MIC values ranging from 2000 to 4000µg/ml. However the hexane, butanol, ethanol and the aqueous extracts were not found to possess significant activity.

**Key words:** *Thymus serpyllum* · Antibacterial · Antifungal · Medicinal plants

## INTRODUCTION

Traditional medicinal plants have a long history of therapeutic use. A primary advantage of botanicals is their complex composition consisting of collections of related compounds having multiple activities that interact for a greater total activity. Historically, natural products have provided an endless source of medicine. The need of new chemical entities (NCEs) for health care is explored and served through the plant sources. Herbal drugs have gained lot of acceptance in the recent years because they have a relatively higher therapeutic window, less serious side effects and are economical. They are now being extensively studied to find the scientific basis of their therapeutic actions [1].

Despite the development of antibiotics, bacterial and fungal infections are still a major issue in medicine and the presence of numerous drug-resistant strains poses a new challenge. Herbal drugs have been extensively used in this field for many centuries [2]. A wide range of medicinal plants have been found to possess antimicrobial properties [3,4,5].There are countless examples of plants which are used topically and systemically to treat bacterial infections in the ethnobotanical setting [6]. In this regard, plant essential oils may offer a great potential and hope. Various essential oils are biocides against a broad range of organisms such as bacteria, fungi, viruses, protozoa, insects and plants [7,8].

*Thymus serpyllum* commonly known as Jawand in Kashmir and used as a culinary herb, as well as for aromatizing and traditional medicinal purposes. The word thyme is a general name for more than three hundred Thymus species, hybrids, varieties and ecotypes, all of which are small perennial herbs native to Europe and Asia. There are many traditions related to the tonic character of these plants. The Egyptians used it in embalmment. The Roman soldiers used to take a bath in water with thyme to provide vigour. Thyme sprigs were thought to offer protection against the plague and were also burned indoors to cleanse the air. Thyme oil was used as an antiseptic during the first World War, Still today, thyme is used in the embalmment liquids, protects paper from mould and is used to preserve anatomy and botany specimens [9,10,11]. Considering the potentiality of the plant as source of antimicrobial drugs a systematic investigation was undertaken to screen the plant for antibacterial and antifungal activity.

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## MATHERIALS AND METHODS

**Collection of Plant Materials:** The plant *Thymus serpyllum* was collected from Aharbal and Kongdoori area of Gulmarg, Kashmir in the month of May-June and was identified and authenticated by the courtesy of Centre of Plant Taxonomy, Department of Botany, University of Kashmir, J&K, India.

**Solvent Extraction:** The authentically identified plant material was shade dried under room temperature at 30±2°C. The dried material was ground into powder using mortar and pestle and sieved with a sieve of 0.3mm aperture size. The powder obtained was successively extracted in hexane, ethyl acetate, absolute ethanol, methanol, butanol and distilled water by using Soxhlet extractor (60°C -80°C). The extracts were then concentrated with the help of rotary evaporator under reduced pressure and the solid extracts were stored in refrigerator for further use.

**Microbial Strains:** Eight reference strains of the following species were used for their susceptibility to *Thymus serpyllum* in this study: *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 14990, *Enterococcus coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Aspergillus fumigatus* MTCC 1811 and *Aspergillus niger* ATCC 16404. These strains were procured from the American Type Culture Collection (ATCC, Manassas, VA, USA) and Microbial Type Culture Collection (MTCC, Chandigarh, India).

**Determination of Antimicrobial Activity:** The antibacterial and antifungal activities of the *Thymus serpyllum* were performed by broth micro dilution methods as per the guidelines of Clinical and Laboratory Standard Institute (formerly, the National Committee for Clinical Laboratory Standards). Mueller–Hinton broth was supplemented with calcium (25 mg/L) and magnesium (12.5 mg/L) for bacterial strains. The RPMI 1640 medium buffered to a pH of 7.0 with 0.165 M MOPS was used for fungal strains. The minimum inhibitory concentration (MIC) was determined by serial two–fold dilution of the test sample in the respective test medium in amounts of 100 µl per well in 96–well U–bottom microtiter plates (Tarsons, Kolkata, India). The stock inoculum suspensions of the bacteria, were prepared in sterile normal saline (0.85%) containing 0.05 % polysorbate 20 from the overnight cultures grown on Trypticase soy agar and the stock inoculum suspensions of the fungi were prepared in sterile normal saline (0.85%) containing 0.05 % polysorbate 20 from the overnight (7–day for *Aspergillus* species) cultures grown on potato dextrose agar. Inocula were verified for each assay by plating onto agar plates for colony enumeration.

These suspensions were further diluted in the respective mediums and a 100 µl volume of this diluted inoculum was added to each well of the plate, resulting in a final inoculum concentration of 5 $x10^5$ CFU/ml for bacteria, 0.5x $10^4$ to 2.5 $x10^3$ CFU/ml for *Candida* species while as 0.4 $x10^4$ to 5x $10^4$ CFU/ml for *Aspergillus* species (Wayne, 2008a, 2008b, 2008c). The controls comprised of the bacterial and the fungal cells or spores suspended in sterile medium only. The microtiter plates were incubated at 35°C for 24 hrs for bacterial cultures and 48 hrs for fungal cultures. The plates were read visually and the MIC was defined as the lowest concentration of test sample that prevented visible growth with respect to the growth control.

## RESULTS

The present study revealed that ethyl-acetate and the methanolic extracts of *Thymus serpyllum* possesses potential anti bacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus coli*, *Pseudomonas aeruginosa* and anti fungal activity against *Candida albicans*, *Candida parapsilosis*, *Aspergillus fumigatus* and *Aspergillus niger* (Table 1 and 2). The MIC ranged from 1000 to 4000 µg/ml. However the hexane, butanol, ethanol and the aqueous extracts were not found to be significantly active.

## DISCUSSION

In recent years there has been an increasing interest in the use of natural substances and some questions concerning the safety of synthetic compounds have encouraged more detailed studies of plant resources. Plants are rich source of bioactive secondary metabolites of wide variety such as tannins, terpenoids, saponins, alkaloids, flavonoids and other compounds, reported to have in vitro antifungal properties. A series of molecules with antifungal activity against different strains of fungus

Table 1: The inhibition of the microbial growth in presence of the ethyl-acetate extract of *Thymus serpyllum*

| | Bacterial Strains | | | | Fungal Strains | | | |
|---|---|---|---|---|---|---|---|---|
| Dilution Factor | SA | SA | SE | EC | CA | CP | AF | AN |
| 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 2 | 3 | 3 | 2 | 2 | 3 | 3 | 2 | 3 |
| 4 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 |
| 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

The concentration of the stock was 4mg/ml.

The following scale was used: 3, means full inhibition, no growth; 1, means no inhibition, full growth and 2 is the value in between.

SA (*Staphylococcus aureus* ATCC 29213), SE (*Staphylococcus epidermidis* ATCC 14990), EC (*Enterococcus coli* ATCC 25922), PA (*Pseudomonas aeruginosa* ATCC 27853), CA (*Candida albicans* ATCC 90028), CP (*Candida parapsilosis* ATCC 22019), AF (*Aspergillus fumigatus* MTCC 1811) and AN (*Aspergillus niger* ATCC 16404).

Table2: The inhibition of the microbial growth in presence of the methanolic extract of *Thymus serpyllum*

| | Bacterial Strains | | | | Fungal Strains | | | |
|---|---|---|---|---|---|---|---|---|
| Dilution Factor | SA | SA | SE | EC | CA | CP | AF | AN |
| 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 2 | 3 | 3 | 2 | 2 | 3 | 3 | 2 | 3 |
| 4 | 2 | 3 | 2 | 2 | 3 | 3 | 2 | 2 |
| 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

The concentration of the stock was 4mg/ml.

The following scale was used: 3, means full inhibition, no growth; 1, means no inhibition, full growth and 2 is the value in between.

SA (*Staphylococcus aureus* ATCC 29213), SE (*Staphylococcus epidermidis* ATCC 14990), EC (*Enterococcus coli* ATCC 25922), PA (*Pseudomonas aeruginosa* ATCC 27853), CA (*Candida albicans* ATCC 90028), CP (*Candida parapsilosis* ATCC 22019), AF (*Aspergillus fumigatus* MTCC 1811) and AN (*Aspergillus niger* ATCC 16404).

have been found in plants, which are of great importance to humans [4]. These molecules may be used directly or considered as a precursor for developing better molecules. Plants have an almost limitless ability to synthesize aromatic substances of different functional groups, most of which are phenols or their oxygen-substituted derivatives. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects and herbivores.

In the present study the antimicrobial activity of plant *Thymus serpyllum* was evaluated, although the hexane, butanol, ethanol and the aqueous extracts did not show any significant activity, but the ethyl-acetate and the methanolic extracts were found to be active. The antimicrobial activities of many plants can be attributed to the presence of high concentrations of carvacrol, which is known to occur at very high concentrations in many plant oils, including the members of the *Labiatae* family, such as *Thymus, Coridothymus, Satureja* and *Origanum* [12,13,14]. The pharmacological actions of the plant extracts are suggested to parallel to their carvacrol content [15]. Carvacrol is considered to be biocidal, resulting in bacterial membrane perturbations. Furthermore, carvacrol might cross the cell membranes, penetrate the interior of the cell and interact with intracellular sites critical for antibacterial activities [16,17]. Another major component of the plant extracts, *p*-cymene, which is precursor of carvacrol, is a very weak antibacterial, but it probably acts synergistically with carvacrol by expanding the membrane, which results in the destabilisation of the membrane [18].

## CONCLUSION

The present study confirms that the plant *Thymus serpyllum* possesses significant antimicrobial activity. These extracts can be further screened and evaluated for the presence of pure compounds which may be used directly or as a precursor for development of new antibiotics.

## REFERENCES

1. Gupta, Y.K., S. Briyal and A. Gulati, 2010. Therapeutic Potential of Herbal Drugs in Cerebral Ischaemia. Indian J. Physiol Pharmacol., 54(2): 99-122.
2. Srivastava, J., J. Lambert and N. Vietmeyer, 1996. Medicinal plants: An expanding role in development. World Bank Technical Paper. No. 320.

3. Govindarajan, R., M. Vijayakumar, M. Singh, C.H.V. Rao, A. Shirwaikar, A.K.S. Rawat, 2006. Antiulcer and antimicrobial activity of *Anogeissus latifolia.* J. Ethnopharmacol., 106: 57-61.
4. Kambizi, L. and A.J. Afolayan, 2008. Extracts from. *Aloe ferox* and *Withania somnifera* inhibit *Candida albicans* and *Neisseria gonorrhoea.* African J. Biotechnol., 7: 12-15.
5. Mahesh, B. and S. Satish, 2008. Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens.World Journal of Agricultural Sciences, 4(S): 839-843.
6. Magassouba, F.B., A. Diallo, M. Kouyaté, F. Mara, O. Mara and O. Bangoura, 2007. Ethnobotanical survey and antibacterial activity of some plants used in Guinean traditional medicine. J Ethnopharmacol. 114: 44-53.
7. Janssen, A.M., J.J.C. Scheffer and A. Baerheim Svendsen, 1987. Planta Med. 53, 395.
8. Deans, S.G., 1991. In *Essential Oils and Waxes*, Linskens, H.F., Jackson, J.F. Eds. Springer-Verlag, 2: 309-320.
9. Kruger, A., 1992. An illustrated guide to herbs their medicine and magic. Dragon's World, London.
10. Morales, R., 2002. The history, botany and taxonomy of the genus. In: Stahl-Biskup E, Sáez F, Eds. Medicinal and Aromatic Plants – industrial profiles Taylor & Francis, London, 17: 1-43.
11. Zarzuelo, A. and E. Crespo, 2002. The medicinal and non-medicinal uses of thyme. In: Stahl- Biskup E, Sáez F, Eds. Medicinal and Aromatic Plants – industrial profiles. Taylor & Francis, London, 17: 263-92.
12. Chorianopoulos, N., E. Kalpoutzakis, N. Aligiannis, S. Mitaku, G.J. Nychas and S.A. Haroutounian, 2004. Essential oils of *Satureja, Origanum* and *Thymus* species: Chemical composition and antibacterial activities against foodborne pathogens. J. Agric. Food Chem., 52: 8261-8267.
13. Sokmen, A., M. Gulluce, H.A. Akpulat, D. Daferera, B. Tepe and M. Polissiou, 2004. The *in vitro* antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. Food Control., 15: 627-634.
14. Bounatirou, S., S. Smiti, M.G. Miguel, L. Faleiro, M.N. Rejeb and M. Neffati, 2007. Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian *Thymus capitatus* Hoff. Et Link. Food Chem., 105: 146-155.
15. Aydin, Y., O. Kutlay, S. Ari, S. Duman, K. Uzuner and S. Aydin, 2007. Hypotensive effects of carvacrol on the blood pressure of normotensive rats. Planta Med., 73: 1365-1371.
16. Cristani, M., M. D?Arrigo, G. Mandalari, F. Castelli, M.G. Sarpietro and D. Micieli, 2007. Interaction of four monoterpenes contained in essential oils with modelmembranes: Implications for their antibacterial activity. J. Agric. Food Chem., 55: 6300-6308.
17. Ultee, A., E.P.W. Kets and E.J. Smid, 1999. Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. Appl Environ Microbiol., 65: 4606-4610.
18. Ultee, A., M.H.J. Bennink and R. Moezelaar, 2002. The phenolic hydroxyl group of carvacrol is essential for action against the foodborne pathogen *Bacillus cereus*. Appl. Environ. Microbiol., 68: 1561-1568.